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Spectrum and Detection Rate of *L1CAM* Mutations in Isolated and Familial Cases With Clinically Suspected L1-Disease

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Mutations in *L1CAM*, the gene encoding the L1 neuronal cell adhesion molecule, lead to an X-linked trait characterized by one or more of the symptoms of hydrocephalus, adducted thumbs, agenesis or hypoplasia of corpus callosum, spastic paraplegia, and mental retardation (L1-disease). We screened 153 cases with prenatally or clinically suspected X-chromosomal hydrocephalus for *L1CAM* mutations by SSCP analysis of the 28 coding exons and regulatory elements in the 5′-untranslated region of the gene. Forty-six pathogenic mutations were found (30.1% detection rate), the majority consisting of nonsense, frameshift, and splice site mutations. In eight cases, segregation analysis disclosed recent de novo mutations. Statistical analysis of the data indicates a significant effect on mutation detection rate of (i) family history, (ii) number of L1-disease typical clinical findings, and (iii) presence or absence of signs not typically associated with *L1CAM*-disease. Whereas mutation detection rate was 74.2% for patients with at least two additional cases in the family, only 16 mutations were found in the 102 cases with negative family history (15.7% detection rate). Our data suggest a higher than previously assumed contribution of *L1CAM* mutations in the pathogenesis of the heterogeneous group of congenital hydrocephalus. Am. J. Med. Genet. 92:40–46, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS: X-linked hydrocephalus; DNA diagnostics; L1CAM; mRNA; genetic counseling; de novo mutations

INTRODUCTION

The *L1CAM* gene encodes the neural cell adhesion molecule L1. Mutations in *L1CAM* may lead to an X-chromosomal recessive disorder with main manifestations including hydrocephalus, adducted thumbs, spastic paraplegia, hypoplasia or agenesis of corpus callosum, and mental retardation (L1-disease). X-chromosomal hydrocephalus due to *L1CAM* mutations was assumed to account for approximately 5% of children with congenital nonsyndromal hydrocephalus [Schrander-Stumpel and Fryns, 1998]. Following the first mutation report [Rosenthal et al., 1992], over 100 families and isolated cases with *L1CAM* mutations have been described [L1CAM Mutation Web Page: Van Camp et al., 1996; Bateman et al., 1996; the Human Gene Mutation Database: Krawczak and Cooper, 1997]. Although the majority of reports confirms *L1CAM* as a major gene for X-linked hydrocephalus, less is known about the actual detection rate of *L1CAM* mutations in cases with clinically suspected L1-disease. Detection rate by analysis of a single gene may depend on various factors as the technique applied, genetic heterogeneity of the trait, frequency of phenocopies, and—in the case of de novo mutations—availability of sample material from index cases. Furthermore, in small families or isolated cases it is not possible to reliably prove co-segregation of the disease phenotype and the mutation. Here we present a comprehensive analysis of mutation detection rate in *L1CAM* in prenatally or clinically suspected L1-disease. For some cases data are presented on a functional analysis of *L1CAM* mutations at the mRNA level.

MATERIALS AND METHODS

Patient Samples

The 153 tissue samples from index cases analyzed in our series include chorionic villi, amniotic fluid, fetal fibroblasts, skin fibroblasts, and peripheral blood. The following phenotypic findings were classified as *L1CAM* "typical": hydrocephalus, adducted thumbs, spastic paraplegia, mental retardation, agenesis or hy-

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poplasia of corpus callosum. Other findings, e.g., cleft palate, brain hemorrhage, metabolic disorder, heart malformation, esophageal atresia or club feet, typically not associated with L1-disease, were classified as "atypical."

DNA Analysis

In all cases where a sufficient number of family members was available, segregation analysis was performed using DXS52, DXS15, BGN, and F8C. PCR and SSCP analyses of the 28 coding exons (exons 1b-28) were performed in all cases as described previously [Bunge et al., 1996; Gu et al., 1997]. In addition to the coding regions, the recently described 5′-noncoding regions containing the human sites homologous to murine HPD (located upstream of the first coding exon, i.e., in intron 1a) and NRSE (located downstream of the first coding exon, i.e., in intron 1b) elements [Meech et al., 1999] were also screened. Numbering of *L1CAM* exons, cDNA nucleotides, and amino acids is according to the nomenclature in the *L1CAM* Mutation Web Page [Van Camp et al., 1996].

Reverse Transcription (RT)-PCR

Total RNA was isolated from cultured skin fibroblasts of cases HC68, 70, 112, and 162, and peripheral blood leukocytes (PBL) of the mothers of HC109 and HC120 by RNeasy Mini Kits (QIAGEN) according to the manufacturer's recommendations. Reverse transcription was performed at 42°C for 1 hr by SuperScript™ II (Gibco BRL) and oligoT_{22} or random hexamers as primers. The cDNA pool was used subsequently for further PCR reactions. *L1CAM* cDNA specific primers for PCR and sequencing are listed in Table I.

Analysis of In Vitro Splicing

Genomic *L1CAM* DNA constructs containing intronic and exonic sequences from HC-cases and control individuals were cloned into pSPL3 vector (Gibco BRL), transfected in COS7 cells using FuGENE transfection reagent (Roche). Splicing products were analyzed by gel electrophoresis after RT-PCR using vector-specific primers. Forty-eight hours after transfection total cellular RNA was extracted.

TABLE I. Sequences of Human *L1CAM* cDNA-Specific PCR Primers

Name	Sequence (5′ → 3′)	PCR product size (bp)
1L78	AAGCTTATGGTCGTGGCGCTGCGGTACGT	556
1L552	CGCTCGTCCTGCTTGATGTG	
1L286	CGCCCCACTCTGGCTCCTTC	793
1L1045	GTCCAGGCGGGCAGTCTCTC	
1L826	TTCCCACGCCCACCATCAAAT	1005
1L1812	GCCCTACTCTCCACCACATC	
1L1767	TACAGCGACCAGGGCAACTAC	574
1L2322	ACCAGGAAGGGGTCGCTGAC	
1L2181	GTGAAGGGGGAAGGAAATGAG	582
1L2744	CTGGGGTGCTGAAGGTGAAC	
1L2594	GAGGAAGCACAGCAAGAGACA	512
1L3086	CACCCGCTGTGGCTGAGATG	
1L3037	TACGGGAAGGAGGCACTATGG	630
1L3633	TCCACGCTGCCCCCATAATC	
1L3417	CTGCTCCTCGTCCTGCTCATC	715
1L4097	CCAGTCAGGGAGCAAGAAAG	

Sequencing

Purified PCR products were sequenced by Taq dye dideoxy terminator sequencing and an automated sequencer (Perkin-Elmer Applied Biosystems, System 310).

RESULTS

Mutation Data and Identification of De Novo Mutations

Table II shows all mutations found in the 153 unrelated cases included in the study. Forty-six mutations are considered as disease causing (30.1% detection rate). Twelve of them were published previously by Gu et al. [1996, 1997]. Three of the remaining 34 mutations have already been found in other, most likely unrelated families, 1108G→A [Ruiz et al., 1995], 1672C→T [MacFarlane et al., 1997] and 550C→T [Fransen et al., 1996]. In our collection, 1672C→T (HC139) occurred de novo during maternal oogenesis. Of the 46 mutations, 8 have occurred recently, their origin could be determined by segregation analysis and was shown to be maternal (n = 2), grandpaternal (n = 4), grandmaternal (n = 1), and greatgrandpaternal (n = 1). The 46 mutations predict premature stop due to nonsense mutations (n = 11) or frameshifts due to small rearrangements (n = 10), loss or inactivation of splice sites (n = 10), amino acid substitutions (n = 14), and in one case loss of an amino acid.

In HC110 and HC111, respectively, a stop and a frameshift mutation was identified in exon 2. This exon encodes 5 amino acids and is known to be translated in the neuronal splice variant of *L1CAM*. No mutation was found in the regulatory 5′-regions analyzed.

Mutation Detection Rates

Figure 1 shows grouping of all 153 cases in 9 categories depending on family history (only index case, 2 cases, >2 cases) and number of findings (atypical finding together with typical ones [*], 1 typical finding, >1 typical finding). Mutations were found in 16 from 102 (15.7%) cases with negative family history for L1-disease (Groups 1–3 in Fig. 1), in 7 from 20 (35.0%) with one additional case in the family (Groups 4–6 in Fig. 1), and in 23 from 31 (74.2%) with at least two additional cases in the family (Groups 7–9 in Fig. 1; $\chi^2 = 39.0$, $df = 2$, $P < 0.00001$; Mantel-Haenszel test for linear association: $\chi^2 = 37.9$, $df = 1$, $P < 0.00001$). All 7 mutations in Groups 4–6 were found among sibling cases (n = 16).

Mutation detection rate was related to the absence of atypical findings. Mutations were found in only 3 of 45 cases (6.7%) with suspected L1-disease and an atypical finding (Groups 1, 4, and 7 in Fig. 1), in contrast to 43 mutations in 108 cases (39.8%) with no atypical finding

TABLE II. Mutation Data From 53 Samples*

A. Missense mutations (including a single amino acid deletion)

HC No.	Group (Fig. 1)	Reported findings in index case[a]	Age[b]	DNA alteration	Predicted effect	Conservation[c] A	R	M	C	Z	G	D	Ref.
1	9	HC, AT	?	1576delGCA	ΔS526	S	S	S	S	N	D	N	Gu et al., 1997
5	8	HC		1624T → C	S542P	S	S	S	T	T	T	A	Gu et al., 1997
7	9	HC, AT, SP	5	719T → C	P240L	P	P	P	P	A	P	H	Gu et al., 1996
11	8	HC	9	2254G → A	V752M	V	V	V	V	V	I	V	Gu et al., 1997
19	9	HC, AT		541A → G	Y194C	Y	Y	Y	Y	Y	Y	Q	Gu et al., 1996
25	9	HC, MR?	20?	1445T → C	L482P	L	L	L	L	L	L	L	Gu et al., 1997
29	2	HC	5	2222T → C	M741T	M	M	M	Q	R	R	I	Gu et al., 1997
40	7	HC	1	550C → T	R184W	R	R	R	R	R	R	R	
59	2	HC		1223A → T	N408I	N	N	N	N	N	N	N	
91	6	HC, AT	0	2071G → A	A691T	A	A	A	A	A	G	A	
92	5	HC	0	1005G → T	W335C	W	W	W	W	W	W	F	
95	3	HC, CC, AT	1	1262T A	V421D	V	V	V	V	V	V	V	
129	7	HC	0	2252G → C	R751P	R	R	R	R	K	K	Y	
154	2	HC	0	1490G → A	C497Y	C	C	C	C	C	C	C	
160	2	HC?	0	1108G → A	G370R	G	G	G	G	G	G	G	

B. Nonsense mutations

HC No.	Group (Fig. 1)	Reported findings in index case[a]	Age[b]	DNA alteration	Predicted effect	Ref.
10	8	HC	10	828G → A	W276X	Gu et al., 1996
18	9	HC, AT, SP, MR	14	1318C → T	Q440X	Gu et al., 1997
32	9	HC(S), AT	1	3124C → T	Q1042X	Gu et al., 1997
35	9	HC, AT	0	316C → T	Q106X	
86	2	HC	0	1267C → T	Q423X	
106	9	HC, MR, AT	40	3496C → T	R1166X	
110	6	HC, AT, MR	?	79G → T	E27X	
120	2	HC	0	2746G → T	G916X	
121	8	HC	0	614C → A 615C → G	S205X	
139	2	HC	0	1672C → T	R558X	
167	9	HC(S), SP, AT, MR	4	1786G → T	E597X	

C. Frameshift (FS) mutations

HC No.	Group (Fig. 1)	Reported findings in index case[a]	Age[b]	Alteration	Predicted effect	Ref.
8	3	HC, AT	13	52insC	FS 18	Gu et al., 1996
14	5	HC	0	955delG	FS 319	Gu et al., 1996
72	2	?	11	2310delC	FS 770	
76	9	HC, AT	0	3164delAG	FS 1055	
96	8	HC(S)	2	666delG	FS 222	
111	8	?	?	84insA	FS 28	
145	3	HC, AT	0	2889delG	FS 963	
146	1	HC, AT	0	1979delA	FS 660	
162	6	HC, AT, SP, MR	5	1666delG	FS 556	
169	9	HC, AT, CC	0	3452del4	Y1151X	

D. Splice site mutations

HC No.	Group (Fig. 1)	Reported findings in index case[a]	Age[b]	DNA alteration
41	9	HC, AT	16	198 – 2a → t
51	3	HC(S), AT, MR	2	1546 + 2t → c
53	3	HC, AT	1	524 – 1g → a
57	6	HC, MR, AT	10	991 + 2t → c
62	9	HC, AT	0	2208 – 2a → g
97	9	HC, AT		992 – 1g → a
104	3	HC, AT	0	3530 + 3a → t
107	8	HC, AT	0	2431 + 1del gt
158	5	HC	0	197 + 5g → c
163	3	HC(S), AT, MR	4	806 + 1g → c

(*Continued*)

reported (Groups 2 , 3, 5, 6, 8, and 9 in Fig. 1; $\chi^2 = 16.6$, $df = 1$, $P = 0.00005$). Of these 108 cases, mutation detection rate was directly related to the number of L1-typical features reported: 18/63 cases (28.6%) with only one vs. 11/23 cases (47.8%) with two and 14/22 (63.6%) with more than two L1-typical findings ($\chi^2 = 9.1$, $df = 2$, $P = 0.0103$; Mantel-Haenszel test for linear association: $\chi^2 = 9.0$, $df = 1$, $P = 0.003$). In the

TABLE II. (Continued)

E. Mutations of unknown significance and polymorphisms

HC No.	Reported findings in index case[a]	Age[b]	Alteration	Predicted effect	Conservation[c]						
					A	R	M	C	Z	G	D
43	HC, AT	0	523 + 12c → t								
68	HC	1	1704 – 17del tctg								
70	HC, AT	2	523 + 12c → t								
88	MR	10	591T → C	N197							
109	HC	0	875T → C	A285							
110	HC, AT, MR	?	88C → A	H30N		H	H	D			
138	HC	0	197 + 17g → a								
148	HC, AT	0	524 – 12t → a								
154	HC	0	3716G → A	G1239E	G	G	G	A	R	N	
167	HC(S), SP, AT, MR	4	2215C → T	R793W	R	R	R	P	D	E	P

*In three cases, in addition to a pathogenic mutation (listed in part A–D) a second sequence alteration was identified (included in part E).
[a]HC, hydrocephalus; (S), shunt; AT, adducted thumbs; SP, spastic paraparesis; MR, mental retardation; CC, hypoplasia or agenesis of corpus callosum.
[b]Age of oldest mutation carrier the family; 0, less than one year of age or prenatal diagnosis.
[c]A; African green monkey; R, rat; M, mouse; C, chicken; Z, zebrafish; G, goldfish; D, fruit fly.

group of isolated cases (n = 102; Groups 1–3 in Fig. 1), mutations were found only in 1/35 cases (2.9%) with atypical (cleft palate and eye disorder in addition to hydrocephalus and adducted thumbs), and in 15/67 cases (22.4%) with no atypical findings ($\chi^2 = 6.6$, $df = 1$, $P = 0.01002$).

Figure 2 represents a post hoc pooling of the 9 groups categorized in Figure 1, demonstrating mutation detection rates as low as 0.02 in families with no more than two cases and with atypical findings accompanying suspected L1-disease (Groups 1, 4), 0.24 (Groups 2, 3, 5), 0.59 (Groups 6–8), and a detection rate of 0.93 in families with more than two cases and more than one L1-typical finding (Group 9; $\chi^2 = 54.4$, $df = 3$, nominal $P < 0.00001$; Mantel-Haenszel test for linear association: $\chi^2 = 52.9$, $df = 1$, nominal $P < 0.00001$).

Phenotype to Genotype Correlation

Complete loss of L1 function and a tendency toward a severe phenotype and early mortality has been observed in association with extracellular truncating L1 mutations as reviewed by others [Fransen et al., 1998; Yamasaki et al., 1997]. A few exceptions from these predictions, however, have also been observed in our collection. In families HC8, 10, 18, and 72, presenting with extracellular truncating mutations, we identified patients aged more than 10 years whereas in families HC10 and 18, there were also male cases of stillbirth and neonate death [Gu et al., 1996, 1997]. It seems likely that despite the intracellular truncating mutation (R1166X), extracellular functions of L1 remained in part preserved in HC106, a 40-year-old patient with mild hydrocephalus, mental retardation, and adducted thumbs. This patient is the oldest known mutation carrier in our sample. On the other hand, in Family HC169, segregating for an intracellular 4 bp deletion (3452del4) predicting an immediate stop at the mutation site (Y1151X), we first identified the mutation in a

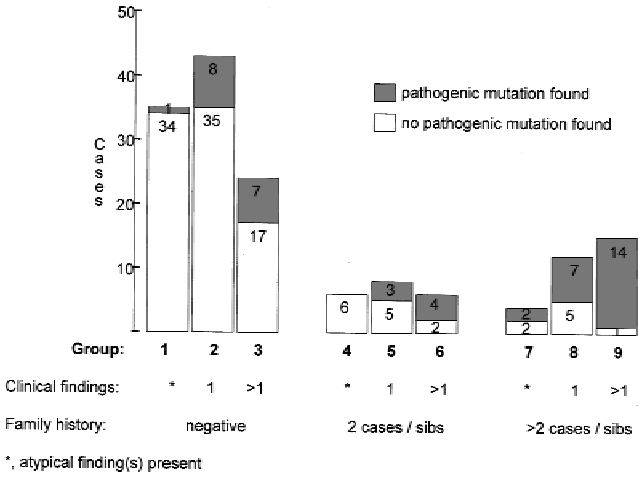

Fig. 1. Numbers of pathogenic mutations detected in cohorts stratified according to the number of reported clinical findings, presence of findings not typically associated with L1-disease and number of affected cases in the respective families.

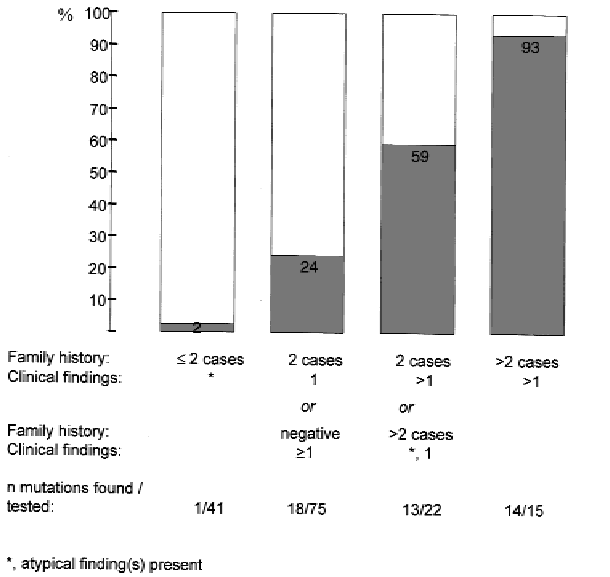

Fig. 2. Mutation detection rates in % relative to family history, number of reported clinical findings, and presence of findings not typically associated with L1-disease.

patient with prenatally diagnosed hydrocephalus that needed shunt operation at postnatal week 3. The patient's mother also carried the mutation, her 36-year-old brother presented reportedly with "spasticity," and both of her sisters have a son with hydrocephalus (14 and 17 years old). The younger of these nephews also had shunt operation and was mentally retarded, whereas the 17-year-old one had hydrocephalus and adducted thumbs. A similar mutation (3453del4) that also predicts Y1151X was found in two independent families [Fransen et al., 1997]. Unfortunately, no clinical details were reported and this mutation was not included in a subsequent genotype-phenotype study [Fransen et al., 1998].

Polymorphisms or Mutations of Unknown Pathogenic Relevance

In the mother of Patient HC154, who died shortly after birth with hydrocephalus, two heterozygous missense mutations were found; C497Y in the fifth immunoglobulin-like domain (Ig5), and a second mutation that predicted an amino acid exchange (G1239E) in the cytoplasmic domain. The latter mutation was also found in her healthy father; the former mutation in her mother. Therefore, G1239E is most likely a nonpathogenic polymorphism. In Patient HC110, a nonsense mutation (E27X) was found in exon 2 together with a mutation predicting H30N shortly downstream of the nonsense mutation. Similarly, in Patient HC167, a nonsense mutation (E596X) in Ig6 was found together with R739W in the second fibronectin III-like domain (Fn2). Due to the assumed truncating effects of the nonsense mutations, H30N and R739W have most likely no specific consequences in these patients. It is unknown, however, whether or not H30N and R739W may be pathogenic on a wild-type background. Two silent mutations and four intronic changes have been detected that do not affect conserved splice site regions and most likely do not introduce novel splice sites. Therefore these variants may have no pathogenic effect. Nevertheless, they seem to be rare or almost unique, because most of them have been observed neither in controls nor in other patients with the exception of 197+17g→a in intron 3 that was found on one of 100 female control chromosomes. Similarly, 523+12c→t in intron 6 was found both in HC43 and HC70. Remarkably, family analysis suggested that 523+12c→t occurred de novo in the grandmaternal oogenesis of HC70 suggesting that it might be pathogenic. Yet, we found no evidence of aberrant splicing in association with 523+12c→t. Several primers that flank the splice junction between exons 6 and 7 yielded RT-PCR-amplification of regular *L1CAM* cDNA fragments from cultured skin fibroblast mRNA of Patient HC70 (data not shown). We did not find evidence of an effect on splicing by the 4 bp deletion 1704-17del4 in intron 14 either. Genomic *L1CAM* DNA constructs containing 27 bp of intron 12, exon 13, intron 13, exon 14, and 44 bp of intron 14 of HC68 and a control individual were cloned into pSPL3 and the transcripts analyzed by RT-PCR. With both constructs we observed very similar splicing patterns with a predominantly regular splicing of exons 13 and 14 (data not shown).

Analysis of *L1CAM* Transcripts

The non-neuronal splice variant of *L1CAM* mRNA that lacks exons 2 and 27 [Reid and Hemperly, 1992; Takeda et al., 1996] was detected in all tissue sources analyzed (PBL, chorionic villi, cultured fetal fibroblasts, skin fibroblasts, and COS7 monkey cell line). Sequencing of RT-PCR products from PBL allowed direct detection of the heterozygous nonsense mutation 2746G→T, predicting G916X in the mother of HC120. In addition, expression of both alleles and no aberrant RT-PCR fragments in PBL were shown by this technique in the mother of HC109 who carried the (assumed) silent mutation 875T→C (A285).

Monkey Homolog

RT-PCR amplification of *COS7 L1CAM* mRNA allowed complete sequencing of African green monkey non-neuronal *L1CAM* splice variant (GenBank accession number: AF129167). Human and monkey *L1CAM* are 96.8% and 99.1% homologous at the cDNA and amino acid level, respectively.

DISCUSSION

Mutations in Neuronal Splice Variants

Exon 2 of *L1CAM* contains 15 nucleotides and is being translated only from the neuronal-specific *L1CAM* splice variant. A frameshift mutation in exon 2 was identified in HC111 and a nonsense mutation in HC110. Although a normal non-neuronal splice variant of L1 may exist in these cases, both HC110 and his brother are mentally retarded, have adducted thumbs, and needed shunt operation due to progressive hydrocephalus. The mutations in these two cases support the notion that human neuronal L1 is of the form that contains the amino acids encoded by exon 2 and that the neuronal-specific splice variant may be essential for normal brain development.

Mutation Spectrum

Our findings are in line with the general observation of a higher proportion of truncating and splice site *L1CAM* mutations that now include worldwide a total of at least 53 truncating mutations, 46 missense mutations, 32 mutations affecting splicing, and 6 in-frame deletions. This may be reflected by the lower proportion of missense mutations (46/137; 34%) compared to a previous report that summarized 16 (47%) missense changes among a total of 34 mutations [Fransen et al., 1996]. This trend parallels the increased number of prenatal samples from families with negative family history or one or more previous male abortions, stillbirths or neonate deaths with hydrocephalus. These "severe" cases contain a lower proportion of missense mutations compared to those surviving and having less severe or no hydrocephalus [Fransen et al., 1996, 1998; Yamasaki et al., 1997]. The majority of our samples (102/153; 66.7%) originated from cases with negative family history for L1-disease. The finding of 16 (15.7%) *L1CAM* mutations in these 102 cases is remarkable, because hydrocephalus is mostly a nonspecific finding or, if inherited, genetically heterogeneous. In this group, the probability of finding a mutation depended on the absence of atypical manifestations. Indeed, 15 of

these 16 mutations were found in the 67 cases without atypical manifestation, a detection rate of 22.4% in isolated male cases. This overall detection rate is in line with predictions from former epidemiological studies. After exclusion of other known causes of congenital hydrocephalus, an X-chromosomal gene mutation was suggested to be the cause of congenital hydrocephalus in up to 25% [Burton, 1979] or 7–27% [Halliday et al., 1986] of affected males.

G1239E in the intracytoplasmic domain of L1 seems to be a nonpathogenic variant as it does not cause disease in a male. This may be the second known nonsynonymous L1 polymorphism, the first being V768I [Gu et al., 1997]. In Patient HC167, the mutation 2215C→T (predicting R739W) was found in *cis* with an upstream nonsense mutation (E597X) that presumably determined the phenotype. R739 is modeled as a non-"key" residue in an Fn2 loop domain [Bateman et al., 1996] and seems to be conserved in mammals only (Table II E). Therefore, R739W may also be a nonpathogenic polymorphism.

The high detection rate of *L1CAM* mutations in cases with positive family history and without atypical manifestation supports the view that *L1CAM* is the major gene for X-linked hydrocephalus. Yet, the majority of our cases is from families with one or more affected male siblings or fetuses in a single generation with an overall low number of siblings. Thus in the families with no *L1CAM* mutation an autosomal recessive disorder similar to L1-disease can not be formally excluded. In addition, some mutations may have escaped detection as our search included some likely regulatory elements in 5′-nontranslated regions in introns 1a and 1b but not the 5′ basal promoter, and the sensitivity of SSCP analysis is not expected to reach 100% [Hayashi and Yandell, 1993].

Mutation Analysis in Transcripts

The feasibility of mRNA-based mutation search by direct RT-PCR sequencing from peripheral blood leukocytes of mothers with recurrent abortions due to suspected severe L1-disease may be important for molecular diagnostics. The finding of a heterozygous nonsense mutation in a carrier mother suggests expression of both the normally spliced *L1CAM* transcript containing the premature stop codon and the wild-type as well as the lack of influence of mutated L1 on the X-inactivation pattern in PBL. However, there are several limitations of this screening strategy: (i) mutations leading to exon skipping or non-amplifiable aberrant transcripts may escape detection in heterozygotes or somatic mosaics; (ii) mutations in neuron-specific exons found in two of our cases are not identifiable in transcripts from PBL specimens; (iii) low somatic or germ line mosaicism or de novo mutations during oogenesis, as found in two of our cases, are not recognizable in transcripts from peripheral blood of carrier mothers.

CONCLUSIONS

In conclusion, we propose a higher than previously assumed incidence of *L1CAM* mutations as cause of unexplained inborn hydrocephalus. Therefore a more liberal use of the DNA-based diagnostics seems to be advisable in severe cases, especially in the patient groups with promising detection rate. Due to the increasing number of mutations with unknown consequence functional assays are required for conclusive molecular diagnostics in L1-disease. The effect of some missense mutations in L1 may be assessed in vitro for the ability to influence neurite growth or to bind various ligands [De Angelis et al., 1999; Zhao and Siu, 1996].

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